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Decreases in Alzheimer's disease like pathology associated with C5aR inhibition correlate with improved performance in a passive avoidance task

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Alzheimer's disease (AD) is the most prevalent cause of neurodegeneration within the elderly population. AD is associated with the accumulation of protein aggregates composed primarily of fibrillar β-amyloid or hyperphosphorylated tau (neurofibrillary tangles). Inflammatory cells and complement proteins are also prominent in AD brain, associated with fibrillar amyloid plaques and/or neurofibrillary tangles. Ablation of C1q resulted in significantly reduced inflammatory glial in the Tg2576 AD mouse model. To assess the contribution of a later component of the complement pathway, the chemotactic peptide C5a, the C5a receptor antagonist (PMX205: cyclo-hydrocinnamate-[Orn-Pro-D-cyclohexylalanine-Trp-Arg]) was administered to 12-month-old Tg2576 animals for a 12-week period via drinking water. Upon completion of drug treatment, immunohistochemical and image analysis demonstrated a 54% reduction in fibrillar β-amyloid (thioflavin positive) area in treated mice ($n=17$) vs. untreated mice ($n=11$) ($p<0.001$). Both total β-amyloid (6E10) and activated microglia (CD45 positive) were also significantly reduced in PMX205 treated mice by 29% and 49%, respectively. The reduction in pathology was correlative with improvements in cognitive performance, as assayed 24 h after an initial foot shock, using a passive avoidance behavioral task with 58 s retention latency for untreated mice ($n=4$) vs. 130 s retention latency for treated mice ($n=5$). Wild-type mice exhibited a 142 s retention in the same task ($n=11$). These results provide evidence that blockade of C5aR in an AD mouse model results in reduced pathology and improved cognition, suggesting a possible therapeutic target for reducing pathology and improving cognitive function in human AD patients.

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Complement anaphylatoxin C5a and C5a receptor are fundamental to neutrophil activation and glomerulonephritis induced by anti-neutrophil cytoplasmic antibodies

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Antineutrophil cytoplasmic autoantibodies (ANCA) cause pauci-immune necrotizing crescentic glomerulonephritis (NCGN) and systemic small vessel vasculitis. Complement participation was recently demonstrated in ANCA-induced NCGN. We tested the hypothesis that the anaphylatoxin C5a is pivotal to disease induction via the neutrophil C5a receptor (C5aR). We demonstrated by ELISA that supernatants from ANCA-activated neutrophil activate the complement cascade in normal serum resulting in C5a production. This conditioned serum primed neutrophils for ANCA-induced respiratory burst as shown by two independent assays. Priming was abrogated by blockade of neutrophil C5aR, but not by C3a receptor blockade. Furthermore, recombinant C5a, but not C3a, primed neutrophils dose-dependently for ANCA-induced respiratory burst. We then tested the *in vivo* role of the C5aR in a mouse model of anti-MPO IgG-induced NCGN in which wild-type (WT) mouse bone marrow (BM) was transplanted into irradiated MPO-deficient mice immunized with MPO. When we used BM from C5aR-deficient (C5aR−/−) mice instead of WT mice, NCGN was markedly reduced. All WT mice (6/6), but only 1/8 C5aR−/− mice developed NCGN ($p<0.05$). The percentage of crescents decreased significantly from 11.3 ± 3.4 in mice transplanted with WT BM to 0.5 ± 0.3 when C5aR−/− BM was used. Albuminuria was 54.0 ± 13.4 μg/ml in the WT mice compared to 10.0 ± 1.5 μg/ml in the C5aR−/− mice ($p<0.05$). In addition, glomerular neutrophil influx was significantly reduced in the C5aR−/− mice, as demonstrated by GR-1 staining. These findings suggest that C5a and the neutrophil C5aR provide an amplification loop for ANCA-mediated neutrophil activation. Thus, the C5aR may provide a new therapeutic target for more specific treatment in ANCA-induced NCGN.

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The receptor for complement anaphylatoxin C5a protects against the development of airway hyperresponsiveness in allergic asthma by inhibiting cysteinyl leukotriene pathway

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We have reported earlier that complement component C5 protects against the development of airway hyper-responsiveness and airway inflammation in a rodent model of *Aspergillus fumigatus* induced allergic airway disease. Our goal for the present study was to determine if these protective responses could be mediated by C5a, which is produced when C5 undergoes activation-induced cleavage. By ablating the receptor for C5a (C5aR), either by genetic deletion or by pharmacological blockade by a specific antagonist, we were able to study the effects mediated by C5a in this disease model.

Consistent with previous results, antigen-challenged C5aR−/− mice and wild-type (WT) mice treated with a specific C5aR antagonist exhibited significantly exacerbated airway hyperresponsiveness (AHR) compared to WT mice. Despite increases in airway responses, there were no significant differences in eosinophil or lymphocyte infiltration into the airways, mucus production or secretion into the airway lumen and total IgE levels. We also examined if Th2 responses, that are known to regulate many aspects of allergic airway inflammation including AHR, were elevated in the lungs of C5aR−/− mice as C5a and its receptor are thought to promote Th1 responses. Surprisingly, Th2-type cytokines IL-5 and IL-13 were significantly reduced in the lungs and bronchoalveolar lavage fluid of C5aR−/− mice. However, pretreatment with a specific cysteinyl leukotriene (cysLT) receptor 1 antagonist could abolish AHR in C5aR−/− as well as in WT mice, suggesting that the absence of C5aR leads to increased cysLT signaling in the lung. In conclusion, deficiency or antagonism of the C5aR in a mouse model of pulmonary allergy causes elevated AHR despite reduction of pulmonary levels of Th2 cytokines IL-5 and IL-13. Furthermore, reversal of AHR by a cysteinyl leukotriene receptor

antagonist, suggests that the absence of the C5aR causes increased AHR by altering leukotriene activity in the lung.

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Pathogen-driven CCR5/C5aR heterodimerization initiates a JNK2/JIP1-dependent signaling pathway that protects from *Toxoplasma gondii* infection

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Microbial recognition is a prerequisite for the development of innate immune responses aimed at warding off infections. Toll-like receptors (TLRs) and C-type lectins of the complement system are cell-bound or soluble pattern recognition systems that have the ability to sense highly conserved microbial motifs. Here we show a novel function for the G-Protein-coupled receptor (GPCR) of C5a, C5aR. We found that C5aR can not only be triggered by its natural ligands C5a and C5adesArg but can serve as a pattern recognition receptor specifically recognizing a highly conserved pathogen-derived protein. This interaction results in enhanced pairing of C5aR with another GPCR, the chemokine receptor CCR5, triggering a unique signaling pathway in dendritic cells required for host defense against parasitic infection. More specifically, we previously found that the chemokine receptor CCR5 recognizes *Toxoplasma gondii*-derived cyclophilin-18 (C18) driving IL-12 production. Here we show that C18 triggers heterodimer formation of CCR5 with C5aR. C18-induced CCR5/C5aR dimerization then activates a signaling pathway involving JNK-interacting protein 1 (JIP1), MAP Kinase Kinase 7 (MKK7) and c-Jun N-terminal Kinase 2 (JNK2) that is essential for C18-driven IL-12 and is not activated by their respective endogenous ligands. The pathophysiologic relevance of these findings is underscored by our data that deficiency of CCR5, C5aR, JIP1 or JNK2 abolished resistance to *T. gondii* infection due to impaired innate and adaptive immune responses. Our data suggest a novel mechanism of pattern-recognition by pairing of two GPCRs resulting in a new platform by which the host can sense danger and trigger adaptive immunity. As GPCRs are the most abundant family of receptors with several thousand members, it is tempting to speculate that microbial sensing by GPCR pairing might provide to the innate immune system the "diversity" that has been formerly assigned only to the adaptive immune system.

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Animal models

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Pathogenic natural antibodies recognizing Annexin IV are required to develop intestinal ischaemia-reperfusion injury and are selected during development in a CR2/CD21-dependent manner

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Intestinal ischaemia-reperfusion (IR) injury is initiated when natural IgM antibodies (Abs) recognize neo-epitopes that are revealed on ischaemic cells. The target molecules and mechanisms whereby these neo-epitopes become accessible to humoral immune recognition are not well understood. Proposing that intestinal epithelial cells (IEC) may carry IR-related neo-epitopes, we used IEC binding assays to screen hybridomas created from splenic, peritoneal and lymph node B cells of unmanipulated wild type C57BL/6 mice. Using this strategy, we identified a novel IgM-producing hybridoma (mAb B4) that reacts with the surface of IEC by flow cytometric analysis. Injection of 25 μg of mAb B4 alone caused intestinal IR injury following mesenteric artery ligation and reperfusion in Rag1−/− mice that are normally protected from this phenotype (1.8 ± 0.42 injury score (IS) for mAb B4 injected to 0.65 ± 0.09 IS for Rag−/− mice, $p < 0.05$). The antigen recognized on Western blot of IEC lysates by mAb B4 was purified and found to be mouse Annexin IV. Pre-injection of 50 μg of recombinant mouse Annexin IV 5 min prior to the reperfusion phase blocked subsequent intestinal IR injury in wild type C57BL/6 mice, confirming the requirement for recognition of this specific protein in order to develop IR injury in the context of a complex natural Ab repertoire. In addition, Complement Receptor 2 deficient (Cr2−/−) mice demonstrated significantly lower levels of anti-Annexin IV natural Abs as compared to Cr2+/+ mice, consistent with previous observations that Cr2−/− mice fail to fully develop the pathogenic subset of natural Abs necessary for intestinal IR injury induction. These data identify Annexin IV as a key ischaemia-related target antigen that is recognized by natural Abs in a pathogenic process required to develop intestinal IR injury *in vivo* and suggest that CR2-dependent recognition of this antigen is necessary for the normal development of natural Ab producing B cells.

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Dextran sulfate reduces ischaemia/reperfusion injury by modulating the activation of complement and the MAPK pathway

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Complement activation augments the inflammatory response through the mitogen-activated protein kinases MAPK pathway (MAPK), which plays an important role in ischaemia/reperfusion (I/R) injury. We recently described that the endothelial cell protectant dextran sulfate (DXS, MW 5000), an inhibitor of comple-